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An evaluation of the Janzen-Connell hypothesis for a dry tropical forest in Guinea-Bissau

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An evaluation of the Janzen-Connell hypothesis for a dry tropical forest in Guinea-Bissau

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Preface

This thesis was produced in collaboration with Chimbo Foundation. Chimbo is a Dutch NGO that was established in 2007. Its mission is to guarantee the long-term survival of the Western Chimpanzee in the Boé through community-based conservation efforts. The main research facilities are located in Béli, which is one of the larger villages in the Boé region, Guinea Bissau. I conducted an internship where I made an inventory on food availability for the Western Chimpanzee in five different forests. For the thesis work, additional data on seedlings of the species *Cola cordifolia* was collected.

Abstract

The Janzen–Connell hypothesis states that seedling survival increases with distance from the parent tree, which promotes regular spacing of species within a forest and thus biodiversity. Very few studies have tested this hypothesis in tropical Africa. This paper evaluates the occurrence of Janzen-Connell effects for seedlings and adult trees of the species *Cola cordifolia* in a dry tropical forest in Guinea-Bissau, West Africa. This was done by field observations of naturally occurring seedlings and adult trees. Seedlings further away from the parent tree were found to be significantly larger in size, but less numerous, which is in accordance with the Janzen-Connell hypothesis. For the evaluation of the adult trees, a comparison was made with two other species that are commonly found in the area. All adult trees showed strongly clustered patterns, which is not in accordance with the Janzen-Connell hypothesis. The mixed results show a need to further study the drivers of biodiversity in tropical Africa.

Table of Contents

Introduction

1. Tropical diversity and the Janzen-Connell hypothesis

One of the most debated topics in ecology has been what drives high species diversity in tropical forests (Matthesius et al., 2011). Many theories and hypotheses exist that attempt to explain this phenomenon (Brown, 2014). Currently, there is still no unifying theory that fully explains the high diversity in tropical areas. However, the latitudinal gradient of biodiversity is generally derived from the relationship between the Earth and the Sun. The variation of energy input to the Earth creates a temperature gradient from the tropics to the poles. In turn, temperature influences the rate of metabolism and biological activity, which includes the rates of ecological interactions and coevolution (Brown, 2014). Thus, increased productivity and faster ecological interactions lead to higher biodiversity in the tropics compared to the poles (Brown, 2014).

Currently, biodiversity is on the decline, mainly as a result of anthropogenic land use changes (Cepic et al., 2022). The loss of one species can lead to the extinction of other species, as species often rely on each other for their survival (Cepic et al., 2022). Therefore, understanding the drivers of biodiversity and ecological research is becoming more and more relevant to social and global concerns ("Why biodiversity matters," 2017).

Tropical forests have high species diversity and low numbers of each species represented in the forest, compared to forest in more temperate areas (Janzen, 1970). The Janzen-Connell hypothesis is one of the hypotheses that has been used to explain high biodiversity in tropical forests. The hypothesis is partly based on Paine's (1966) predation hypothesis. The experiment that Paine conducted to test this hypothesis showed that if predators are removed from a biological community, this results in a loss of species diversity (Paine, 1966). This is because the predator prevents one species from dominating the ecosystem. Thus, species diversity is related to the efficiency with which predators prevent monopolisation. When efficiency is high, this will allow several other species to establish and cause an increase in biodiversity. The Janzen-Connell hypothesis applies this principle to plant communities (Janzen, 1970; Connell, 1971). The hypothesis states that seed dispersal is such that the highest density of seeds is around the parent with density decreasing with distance from the parent (fig. 1). The predation on seedlings will be highest around the parent and will decrease with distance. This is because predators accumulate where most of the seeds fall, which is closest to the tree. Thus, seedlings close to the parent are more likely to die due to predation, while seedlings further away are less likely to encounter predation and will therefore more likely persist.When a certain species becomes more common, so does its specialised natural predator. The offspring are then more exposed to predation and suffer higher mortality, leaving room for other species (Adler & Muller-Landau, 2005). This has a positive effect on the biodiversity of an ecosystem by promoting regular spacing of species. One species is therefore not expected to form clusters of several individuals, but rather show a dispersed pattern where adult trees are regularly spaced between other tree species. Possible predators include pathogens, parasites, insects and to a lesser extent seed-eating vertebrates. This is because vertebrates are generally less host-specific, compared to the other predators (Janzen, 1970).

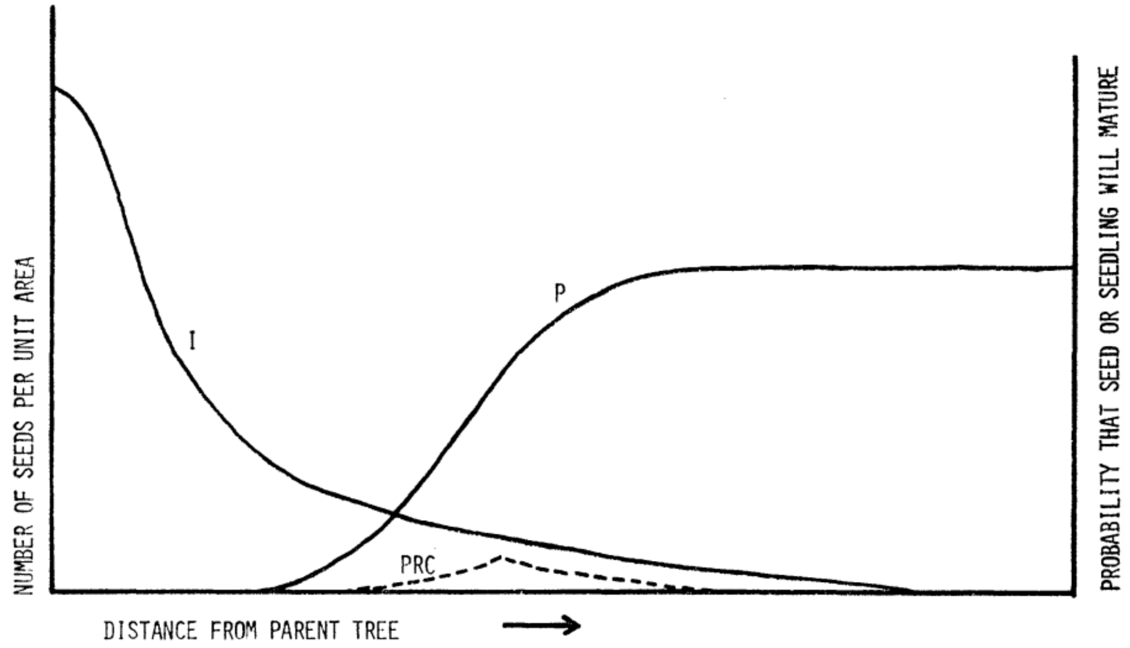


Figure 1. Illustrative figure from Janzen (1970) showing the probability (P) that a seed or seedling will mature as a function of seed-availability, distance from the parent tree, activity of seed and seedling predators. Number of seeds per unit area (I) decreases with distance from the parent tree, while P increases, as a result of reduced chance of predation. Population Recruitment Curve (PRC) is the product of P and I, the area under the curve shows the likelihood that an adult tree will reproduce (Janzen, 1970).

2. Relevance of the hypothesis and previous research

Around 63 papers have been published which have tested the Janzen-Connell hypothesis with varying results (Comita et al., 2014). The papers of Janzen (1970) and Connell (1971) are still highly influential in today's research (fig. 2). However, very few tests have been done in Africa (Comita et al., 2014). A study by Matthesias et al. (2011) evaluated the Janzen-Connell hypothesis for a montane forest in Nigeria, West Africa, which will be used as a comparison in the discussion. Analysis of previous publications shows that Janzen-Connell effects are not observed in all cases (Comita et al., 2014). Studying Janzen-Connell effects in different geographical locations and environments can therefore increase the general understanding on the role of Janzen-Connell effects on tropical biodiversity. Doing multiple studies can show whether Janzen-Connell effects are site or species specific. With that in mind, including an analysis of multiple species in an area is more comprehensive than just studying one single species, which has often been the case in previous research

(Comita et al., 2014). Due to time limitations, this bachelor project focuses on Janzen-Connell effects and seedling survival for one single species. However, comparisons with other species that are present in the forest will be included in the data analysis and discussion.

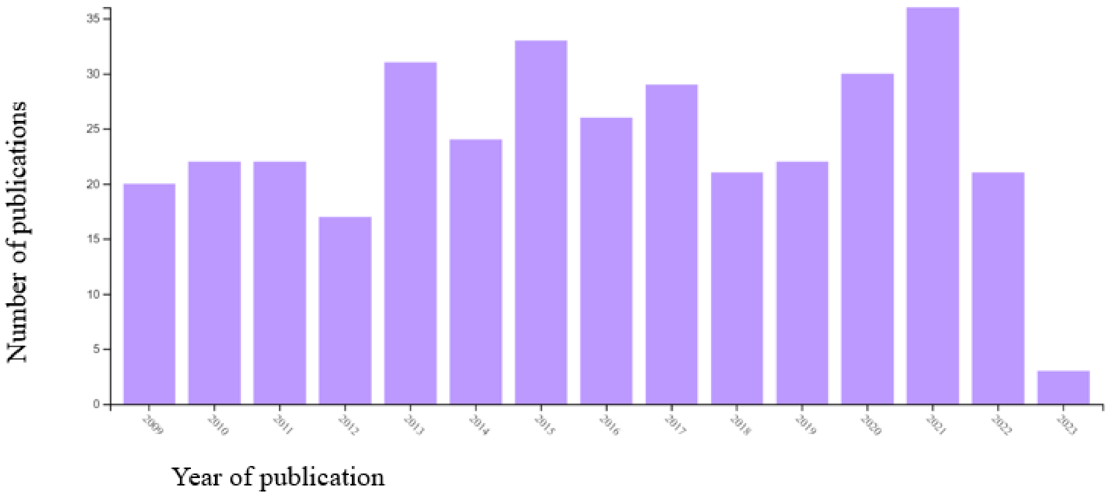


Figure 2. Number of publications citing Janzen (1970) or Connell (1971) between 2009 and 2021 (Web of Science).

3. Objectives and aims

The aim of this thesis is to test whether the Janzen-Connell hypothesis applies to the dispersal and survival of *Cola cordifolia* seedlings and parent trees found in two sacred forests of the Boé region, Guinea-Bissau. Furthermore, by means of spatial analysis it will be tested whether Janzen-Connell effects influence the distribution pattern of individual species in the forest. The question here is whether trees are spatially organised in clumped, dispersed or random patterns.

In summary, the research questions I set out to answer in this bachelor thesis are:

1) Is the distribution of seedlings for the tree species *Cola cordifolia* in accordance with the Janzen-Connell hypothesis? I. e., are seedlings more numerous but smaller in size closer to the adult tree?
2) Is the spatial pattern (clumped, dispersed or random) of adult trees of *Cola cordifolia* consistent with what would be expected according to the Janzen-Connell hypothesis?

Background

1. Study area

Guinea-Bissau is a small country in West Africa, located between Guinea-Conakry and Senegal (fig. 3). This study was carried out in the Boé region, which is found in the South-eastern corner of Guinea Bissau (fig. 3). It is roughly situated between 11°30' and 12°0' northern latitude and between 13°45' and 14°30' western longitude, covering a total area of 3289 km2 (Wit & Reintjes, 1989). Part of the area was declared a national park in 2017 through combined efforts of Chimbo, the Institute for Biodiversity and Protected Areas (IBAP) of the Government of Guinea Bissau and funding from the UNDP/GEF Programme. The local inhabitants continue to inhabit the area, but commercial hunting is not allowed and protected species cannot be targeted in any situation. The region is home to a variety of protected species of both flora and fauna. The species receiving most attention is the endangered Western Chimpanzee (*Pan troglodytes verus*).

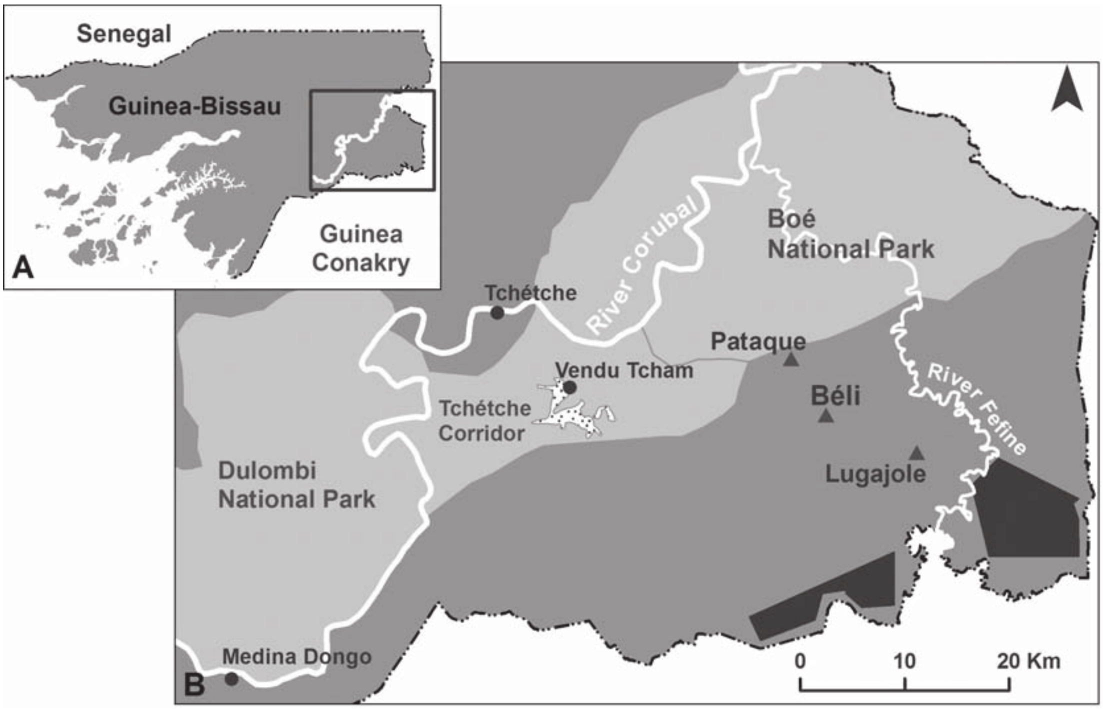


Figure 3. Overview of the Boé region and its location in Guinea-Bissau (Guilherme, 2014).

Most ecosystems in the Boé can be classified as wooded savannah, followed by dry and semi-dry forests, gallery forests and palm groves. Savannah is found where there is a layer of impermeable laterite rock. Forests occur where the soil has a depth of at least 1 metre, or in cracks of the laterite hardpan. Forests that form in the cracks are typically narrow strips of trees up to 20-30 metres in width, which creates a mosaic landscape. The mean annual rainfall is between 1600-2100 mm, with a rainy season lasting from May to November. The mean annual temperature is 28 degrees Celsius (Wit & Reintjes, 1989).

Agriculture and keeping cattle is an important source of food and income for the local population. Agriculture is predominant in the fluviatile soils of the gallery forests, which are quite rare. The population of the Boé is rapidly increasing and therefore the pressure on larger tracts of forest and rare patches of fertile soil is high. Deforestation in an already naturally fragmented area is leading to even more fragmentation. The deforestation of the gallery forests is a big threat to the population of chimpanzees and humans alike, since the local population relies on ecosystem services that the forest provides. More recently, cashew plantations have become an important source of income for the local population, which increases the rate of deforestation (Guilherme, 2014). Other than for commercial uses, most arable land is mainly used for the cultivation of rice and other produce for domestic use. The deforestation of the gallery forests poses a threat to the biodiversity of the region. Therefore, understanding how forests regenerate and maintain diversity is important for nature conservation purposes. Moreover, the natural and anthropogenic fragmentation of the forests of the Boé makes it interesting to study spatial patterns of species abundance. The forests occur in patches that form a mosaic together with savannah grasslands (fig. 4). Until now, no study exists that has tested the Janzen-Connell hypothesis in this type of environment. It is of importance to understand the relevance of Janzen-Connell effects in fragmented forests as globally forests are becoming more and more fragmented as a result of anthropogenic land use change (Liu et al., 2020).

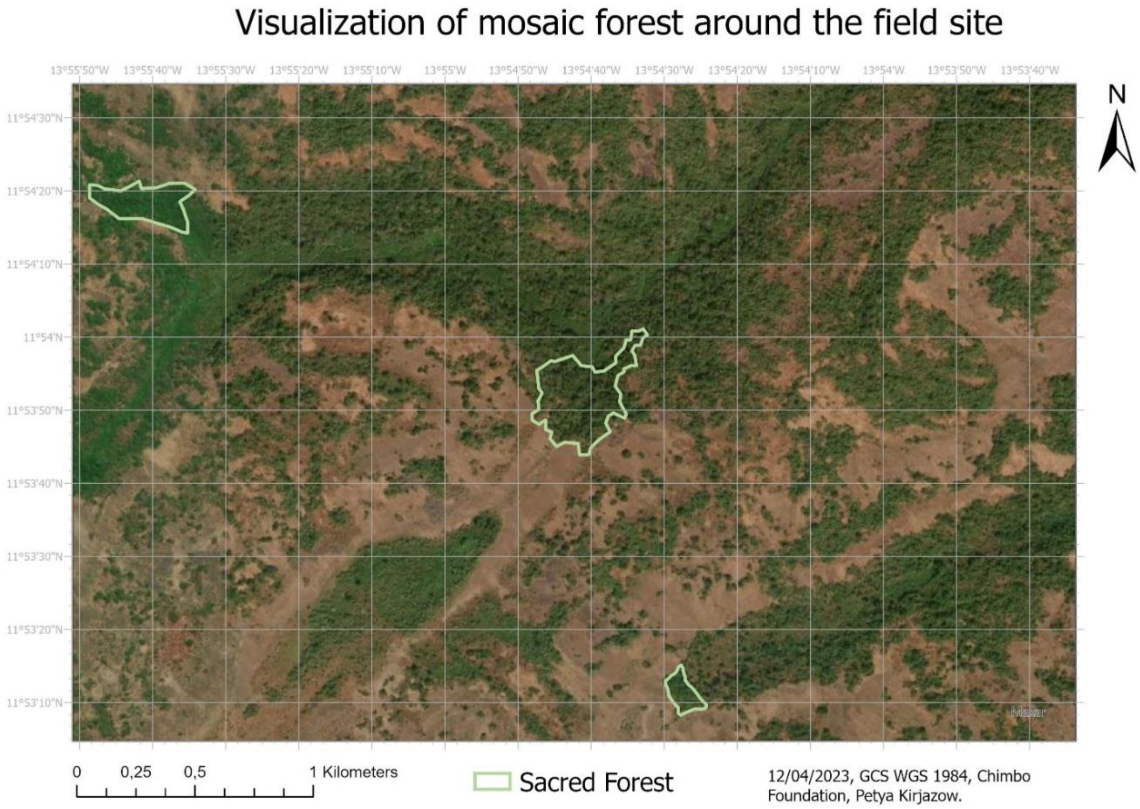


Figure 4. A visualisation of natural fragmentation in the Boé. It creates a mosaic of forest and savannah ecosystems. The map is centred around the sacred forest of Tontege, where most of the data for this study was collected.

2. Sacred forests

Sacred forests are forests which are conserved by local communities, based on the spiritual significance of these places for them (Bhagwat & Rutte, 2006). Sacred forests were chosen for this study as the sacred status means that these forests are relatively undisturbed. Therefore, they could represent the natural state of the forest without too much intervention of humans in the form of logging, hunting, beekeeping, harvesting of fruits and medicine etc., which could disturb the natural processes in the ecosystem. Still, human pressure on sacred forests exists, especially since the area is experiencing population growth and undergoing modernisation which might threaten the traditional stewardship of the sacred forests. Besides sacred forests, the remaining forests of the Boé are partly located in Boé National Park (fig. 3). Despite the protected status, environmental laws are not always enforced by the government of Guinea-Bissau. This is due to the remoteness of the area and lack of monitoring, except for the work that Chimbo does.

3. *Cola cordifolia*

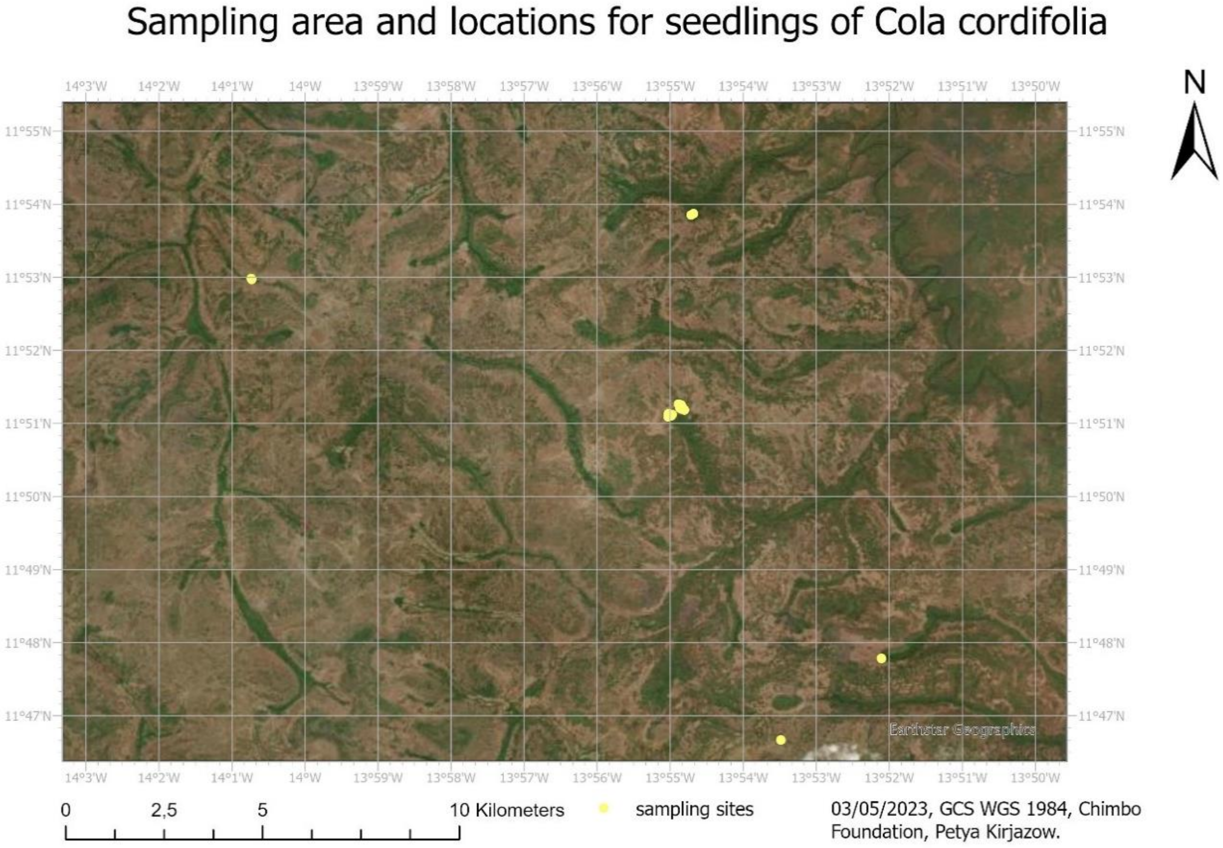
Cola cordifolia is a tree species commonly found in the sacred forests of the Boé area. The tree is characterised by a large, buttressed trunk and a dense crown. It grows up to 15-25 metres high. The species is found in Guinea-Bissau, but also in Senegal and Mali (Grønhaug et al., 2008). *Cola cordifolia* seeds are consumed by chimpanzees, which also use the tree for nest-making (fig. 6). According to the guides from Chimbo, the seeds are dispersed by animals, such as chimpanzees and elephants (personal communication, May 22, 2023). This species was chosen for this study as it commonly occurs in the studied forests (appendix 2). Seedlings were also found abundantly in the forests when the fieldwork was conducted, which was not the case for the other tree species that are found on the study site.

Figure 6. Picture of a chimpanzee nest made in a Cola cordifolia tree somewhere in the Boé, Guinea Bissau.

Methods

1. Data collection of seedlings and adult trees of *Cola cordifolia*

Data collection took place in the months of November and December of 2021, which is the start of the dry season. Data on seedling occurrence and height around parent trees of *Cola cordifolia* was collected in several locations around the Boé (fig. 7, fig. 8). Most of the seedling samples were collected around the sacred forest of Bundu Njuri (fig. 8, top right). In total, 19 parent trees were selected. The two selection criteria were that the trees were mature and not standing right next to another parent tree, to avoid overlapping of canopies and seed rain between two trees.


Sampling area and locations for seedlings of Cola cordifolia

03/05/2023, GCS WGS 1984, Chimbo Foundation, Petya Kirjazow.

Figure 7. Map showing the study area for seedling data collection, with yellow dots showing different locations of parent trees. For each parent tree a sample close and a sample far was collected.

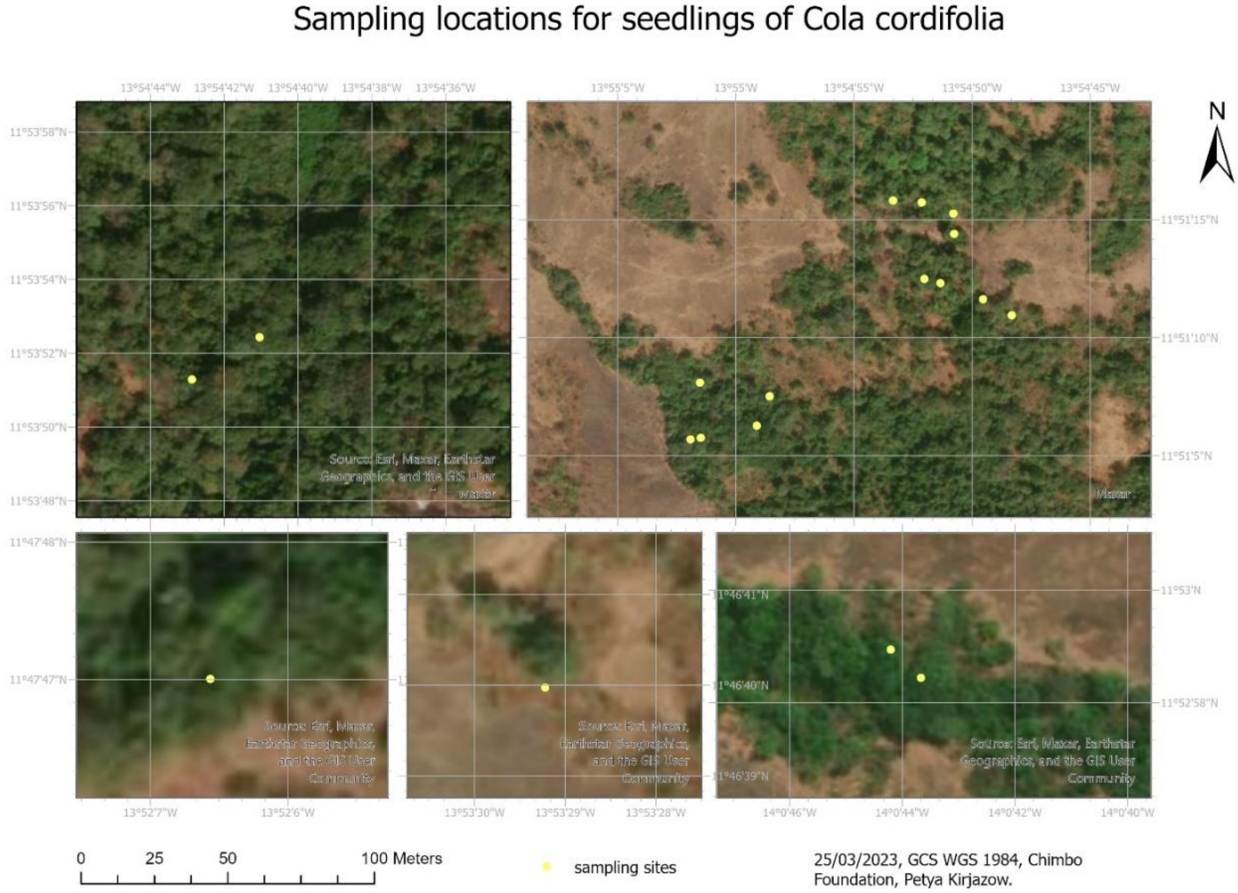


Figure 8. Zoomed in map showing different locations of parent trees. For each parent tree a sample close and a sample far was collected.

For each parent tree a sample close and far was collected. Sampling was done with a square made of sticks that measured 1 metre (fig. 9), as no quadrat was available on site. Samplings close were taken at 5 metres from the parent tree. Samplings far away were taken 9 metres from the parent tree. Based on visual judgement, a difference in seedling abundance could be seen between these two distances. Moreover, these distances were chosen so that samples would be taken under the crown of the parent tree. This was done to have similar light availability for both seedlings close and far. The direction from the parent tree to the sampling location was randomly chosen by turning around with eyes closed until the guide said ‘stop’. This was repeated for each parent tree to sample locations close and far. Once the direction was determined, the distance to the location where the quadrat would be placed was measured with a measuring tape. Then, counts and heights were noted in a notebook. Heights were measured with a measuring tape and noted in centimetres. This was repeated for all samples. If no seedlings were present within the quadrat, location of sampling was not changed, but it was noted that no seedlings were found.

Figure 9. Visualisation of sampling technique with the use of a quadrat.

For the parent trees, data was collected around five sacred forests. Data from two of these forests by the name of Tontege and Bundu Njuri were used for the evaluation of the Janzen-Connell hypothesis. These forests were chosen for the evaluation as they both contained a high number of trees of the species of interest (appendix 2). However, some trees outside of the borders of the sacred forests were also included, and will be taken into account for all data analysis. Therefore, the borders are shown to inform the reader of the location of the sacred forest, but the study area is not limited to these borders. The sacred forests were visited with local Chimbo guides that are based in Béli. They helped with tree identification, navigation through the forest and technical support for the measurements. Scientific names of species were determined with the help of the following book: *Trees, shrubs and lianas of West African dry zones* (Arbonnier, 2004). Some of the scientific names of common trees were found in Chimbo’s database in the office in Béli. Coordinates of the individual trees were taken with a GPS of the model eTrex Touch 25 and is manufactured by Garmin in Olathe, Kansas, USA. The obtained coordinates were then imported to ArcGIS Pro (ESRI, 380 New York Street, Redlands, CA 92373-8100 USA) in order to do further analysis and produce maps of the study sites. All the obtained data were manually transferred from paper into Microsoft Excel. Several allometric parameters were measured for the parent trees, such as height, average crown diameter and diameter at breast height. Height was measured with the use of a clinometer of the model MP-5, manufactured by Suunto in Vantaa, Finland. With known eyeheight, distance to the tree and the angle from the eye to the top of the tree which is measured with the clinometer, tree height could be calculated. Crown diameter was calculated by taking the average of the vertical and horizontal crown diameter, perpendicular to each other. Diameter at breast height was obtained by measuring the circumference of the tree with a measuring tape and then dividing that by pi. The allometric data was not included in the testing of the Janzen-Connell hypothesis but could serve as a useful reference for future research surrounding the topic. Further details on allometric equations and data collection can be found in the field protocol (appendix 1).

2. Data analysis of seedlings

In order to test the Janzen-Connell hypothesis, the collected data was analysed by performing randomisation tests. A randomisation test allows for an unbiased evaluation of the observed results (Suresh, 2011). Randomisation tests randomize the observed data set to produce a distribution of statistical values which can be expected under a defined null hypothesis (Farine & Carter, 2022).

In this case, the null hypothesis states that there is no significant difference between samples close and far, when comparing their height and abundance. The randomisation tests were performed using Microsoft Excel, which allowed for easy visualisation of the separate steps of the randomisation test, and does not require any programming skills. It could be more error prone than other statistical software, since most steps are performed manually. However, care has been taken to verify if equations were correctly executed and if results were coherent. Two separate randomisation tests were carried out to statistically test differences in seedling abundance and height for measurements close and far. For the comparison of the heights one thousand randomisations were performed for each observation using the following equation:

=INDEX($HEIGHTS OF SEEDLINGS CLOSE AND FAR$);RANDBETWEEN(1;ROWS($HEIGHTS OF SEEDLINGS CLOSE AND FAR$));1)

The values in brackets represent the total (close and far) set of observed values for one sample. Randomisations were performed separately for each sample, resulting in 19 randomisations in total. Once randomised, all the randomisations of the 19 samples were pooled together to perform the statistical test. All the randomisations for observations close and far were separated so that the top part of the column represented randomisations close and the lower part randomisations far. Then, the average randomised heights far were subtracted from the average randomised heights close. This was done a thousand times for all randomised observations, using the following equation:

=AVERAGE(RANDOMISATIONS CLOSE)-AVERAGE(RANDOMISATIONS FAR)

To be able to test whether the randomisations were significantly different from the observations, a value of either 1 or 0 was assigned. If the product of the above mentioned equation was smaller or equal to the observation, number 1 would be assigned. Otherwise 0, as can be seen in the equation below.

=IF(AVERAGE DIFFERENCE RANDOMISATIONS CLOSE AND FAR<=$AVERAGE DIFFERENCE OBSERVATION CLOSE AND FAR$;1;0)

Lastly, to calculate a P-value, the product of the previous equation was summed up for all the randomisations and then divided by one thousand.

For the randomisations of the seedling abundance, a similar method was used. This time, instead of using the observed heights, the numbers 0 and 1 were assigned, where 0 represents the seedlings close and 1 the seedlings far. For each parent tree, the total number of both close and far was combined and assigned a randomised value of either 0 or 1.

A thousand randomisations were performed as with the seedling heights. Then, the sum of the randomisations was divided by the total number of observations per sample. This was done one thousand times for all randomised observations. To account for the increased radial area at the further distance of 9 meters from the parent tree, the abundance of seedlings closer to the parent tree was multiplied by $\frac{5}{9}$. This factor allows for a better comparison of seedling abundance of seeds close and far.

Values of 0 and 1 were assigned in a similar way as for the seedling heights:

=IF(RANDOMISATIONS CLOSE AND FAR<=$OBSERVATION CLOSE AND FAR$;1;0)

Lastly, to calculate a P-value, the product of the previous equation was summed up for all the randomisations and then divided by one thousand.

3. Data analysis of adult trees

A spatial analysis was performed to look at the distribution of *Cola cordifolia* parent trees in two sacred forests, Tontege and Bundu Njuri. Besides *Cola cordifolia,* an analysis was also performed for the species *Pterocarpus erinaceus* and *Spondias mombin*, to allow for a comparison of spatial patterns between different species. These species were chosen as they are the most common species that were mapped in the five sacred forests besides *Cola cordifolia* (appendix 2). An additional analysis of all trees that were mapped in the forest was performed, as to be able to compare Janzen-Connell effects between a single species and multiple species.

The spatial analysis for the different species and forests were performed in ArcGIS Pro. Firstly, Thiessen polygons were created by using the point layer which contains the X and Y coordinates for the trees. Thiessen polygons were used as they are an indicator for proximity to the nearest tree. Small polygons indicate a short distance to the nearest tree, while larger polygons indicate a larger distance to the nearest tree. Then, this polygon layer was used as the input for the spatial autocorrelation (Moran's I) analysis.

The spatial autocorrelation generated a report with statistical values, the spatial pattern (clustered, dispersed or random), the Moran's I index and an evaluation of the null hypothesis. The null hypothesis that is assumed by the tool is that the trees are randomly distributed. The

Moran’s I index is an indicator for spatial patterns that has values which fall between -1.0 and +1.0. If the values in the dataset show clustering, the Moran’s I index will be positive. When features show bigger differences in the values, the index will be negative.

Another way of analysing the spatial distribution of the parent trees is by doing a nearest neighbour analysis. This analysis was also carried out in ArcGIS Pro. It gives an indication of the distances between the different parent trees to better understand how trees are spaced within a forest and how this changes for different species.

4. Literature review

A literature review was performed in order to put the results into context and compare results and methods with other studies that have evaluated the Janzen-Connell hypothesis. The literature review also served as a starting point for this thesis, as it allowed for an understanding of the broader context of the Janzen-Connell hypothesis and biodiversity in tropical forests. Search engines that have been used include Web of Science and Google Scholar. Search words included: ‘Janzen-Connell hypothesis’, ‘Guinea-Bissau’, ‘tropical diversity’.

Results

1. Randomisation tests

Randomisations showed that seedlings are significantly (p-value: 0.005) larger in height further away from the parent tree. The randomisation test also showed that seedlings are more numerous close to the parent tree (p-value: 0.001).

2. Mapping of *Cola cordifolia* adult trees in two forests

For the sacred forest of Tontege, the locations of the mapped trees are shown below (fig. 10). In total, 1036 trees were recorded for this forest, of which 269 trees were *Cola cordifolia* trees (fig. 12). Some natural fragmentation can be observed from the map of Tontege. South-west from the sacred forest, for example, there is an area of savannah grassland and then a small patch of trees is found again (fig. 10). For the analysis of the forest of Tontege, only the main body of forest is considered. The two small clusters of trees to the west of the sacred forests were excluded, as these would push the results of the spatial analysis towards clustering.

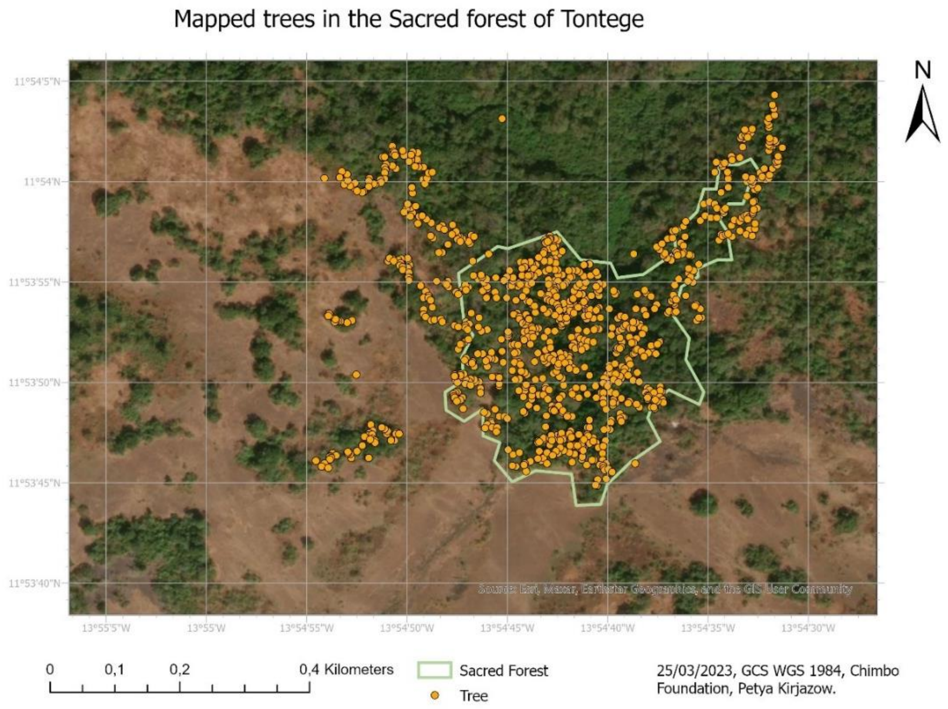


Figure 10. Mapped trees in the area of the sacred forest of Tontege. Each orange point represents an individual tree.

For the forest of Bundu Njuri, 263 trees were mapped in total (fig. 11), of which 77 were *Cola cordifolia* trees (fig. 13). All mapped trees of *Cola cordifolia* that were measured in the forest of Bundu Njuri were considered for the data analysis.

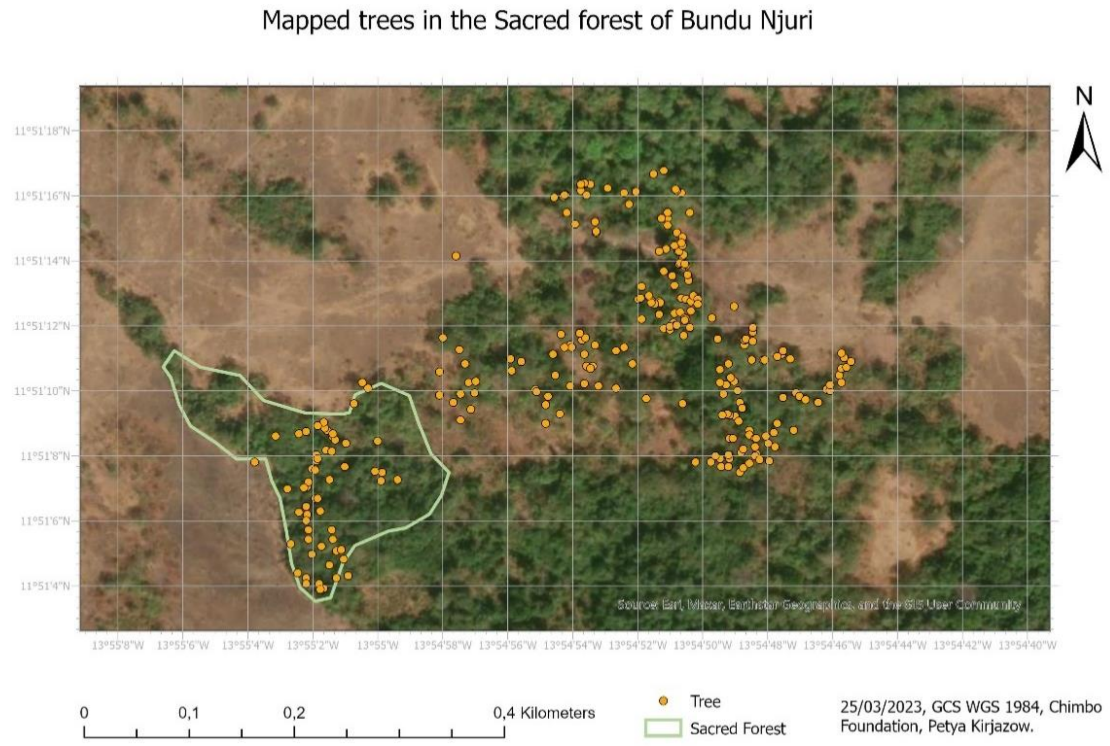


Figure 11. Mapped trees in the area of the sacred forest of Bundu Njuri. Each orange point represents an individual tree.

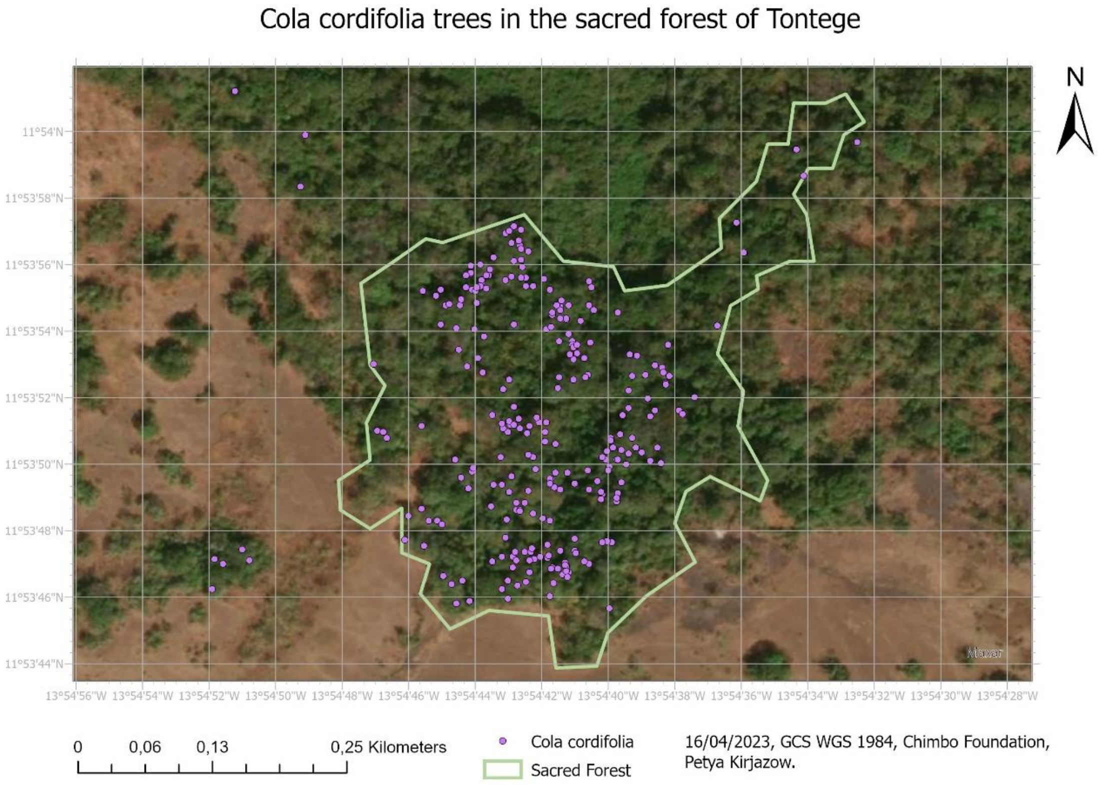


Figure 12. Mapped Cola cordifolia trees in the area of the sacred forest of Tontege. Each purple point represents an individual tree.

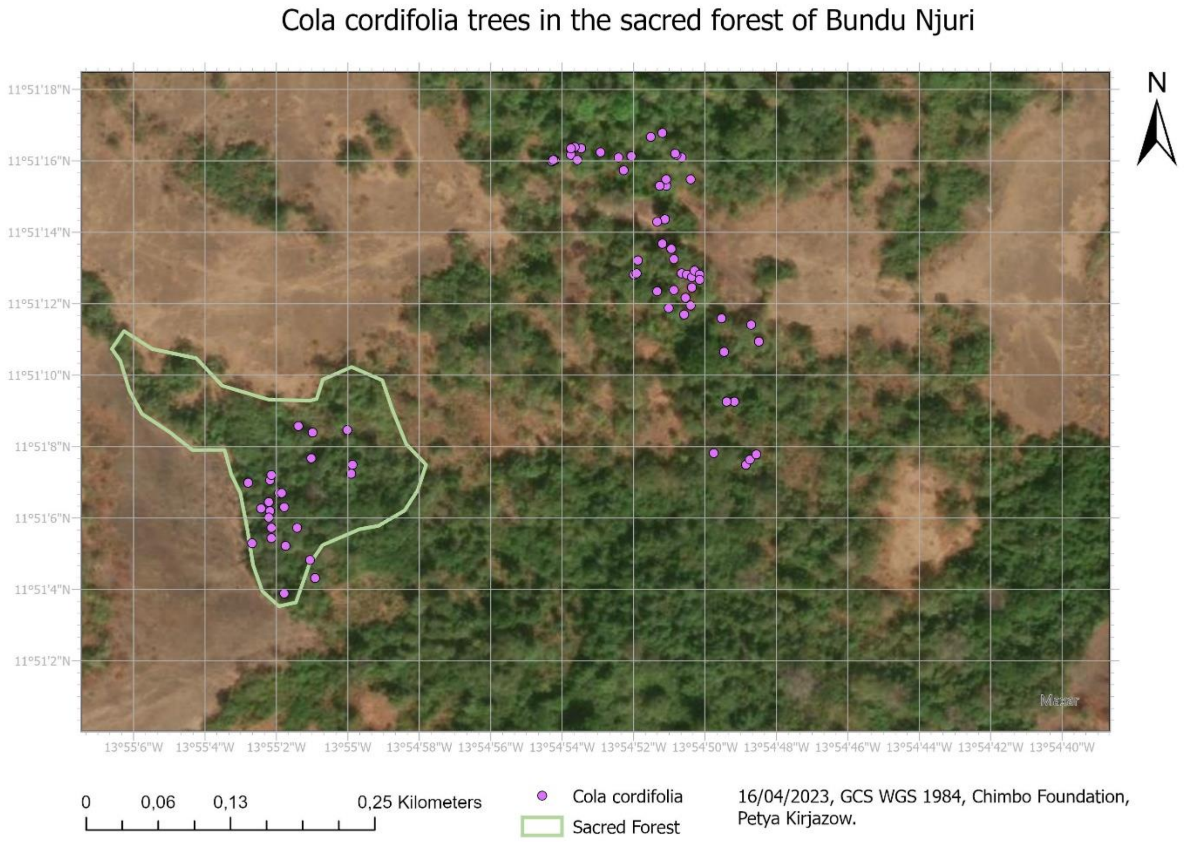


Figure 13. Mapped Cola cordifolia trees in the area of the sacred forest of Bundu Njuri. Each purple point represents an individual tree.

Maps showing the distribution of the Thiessen polygons for both the forests of Tontege and Bundu Njuri are shown below (fig. 14, fig. 15).

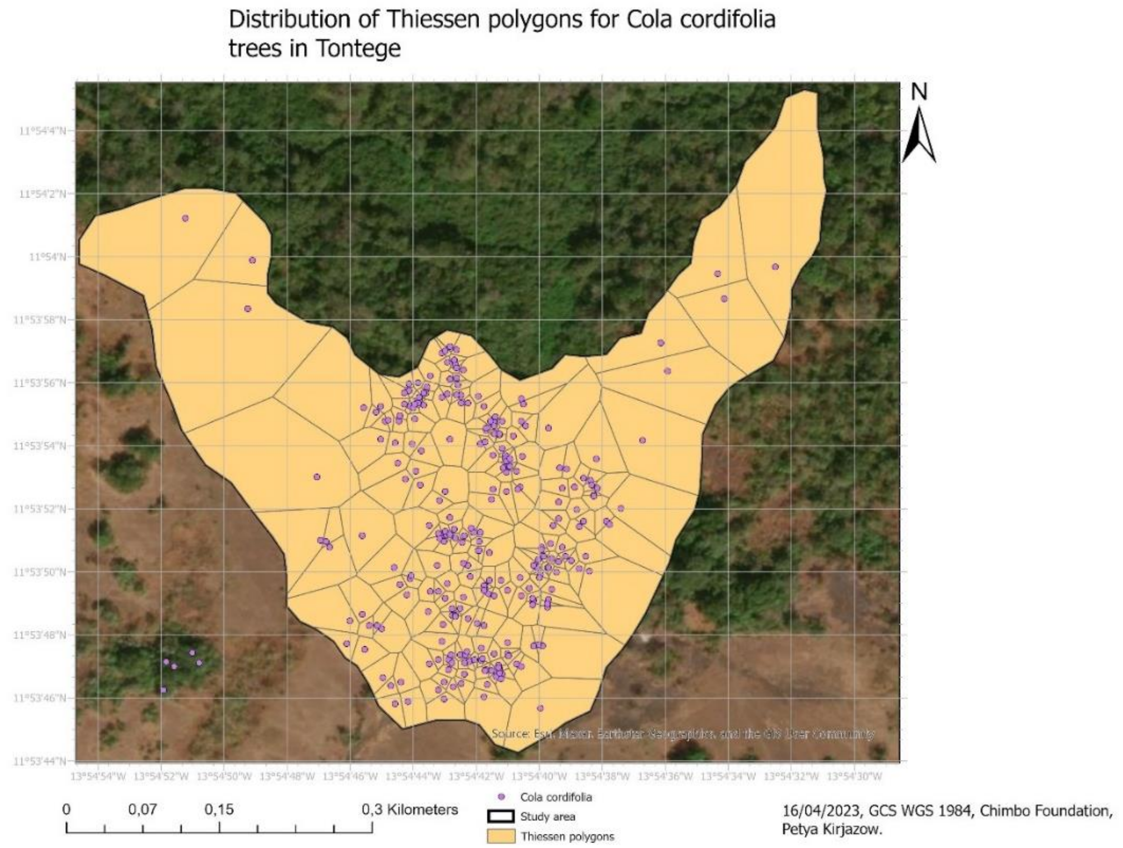


Figure 14. Thiessen polygons based on the distribution of Cola cordifolia adult trees in the sacred forest of Tontege.

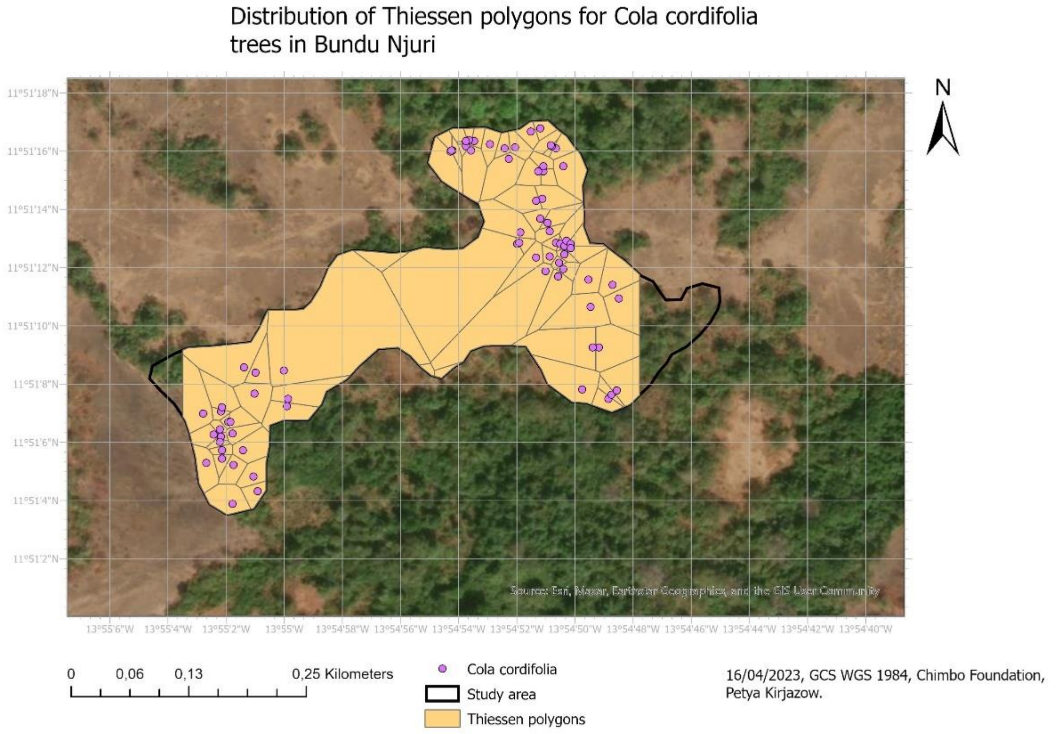


Figure 15. Thiessen polygons based on the distribution of Cola cordifolia adult trees in the sacred forest of Bundu Njuri.

3. Spatial autocorrelation - Moran's I and nearest neighbour analysis

Based on the Thiessen polygons, the spatial analysis shows that all species within the two forests are arranged in clustered patterns (table 1). According to the Moran's I index, *Cola cordifolia* shows the strongest clustering for the forest of Tontege. For the forest of Bundu Njuri, *Spondias mombin* shows the strongest clustering.

Table 1. Results for the spatial analysis (Moran's I) of the forests of Tontege and Bundu Njuri.

Field site	**Species name**	**Moran's I index**	**Clustered, random, dispersed**	**P-value**
Tontege	*Cola cordifolia*	0.391	Clustered	$<10^{-6}$
	Pterocarpus erinaceus	0.025	Random	$5.2 * 10^{-5}$
	Spondias mombin	0.271	Clustered	$<10^{-6}$
	All mapped trees	0.216	Clustered	$<10^{-6}$
Bundu Njuri	*Cola cordifolia*	0.101	Clustered	$2.5 * 10^{-5}$
	Pterocarpus erinaceus	0.246	Clustered	$1.3 * 10^{-5}$
	Spondias mombin	0.400	Clustered	$<10^{-6}$
	All mapped trees	0.200	Clustered	$<10^{-6}$

The average distance to the nearest neighbour shows that there is a difference between species in how strongly they cluster (table 2). Clustering is strongest for the species *Spondias mombin*, followed by *Cola cordifolia*, while the largest distance to the nearest neighbour is observed for the species *Pterocarpus erinaceus* (table 2). This is the case for both forests.

Table 2. Results for the spatial analysis (nearest neighbour) of the forests of Tontege and Bundu Njuri.

Field site	**Species name**	**Average distance to nearest neighbour (metres)**	**Clustered, random, dispersed**	**P-value**
Tontege	*Cola cordifolia*	11.0	Clustered	$<10^{-6}$
	Pterocarpus erinaceus	24.2	Clustered	$<10^{-6}$
	Spondias mombin	9.9	Clustered	$<10^{-6}$
	All mapped trees	5.7	Clustered	$<10^{-6}$
Bundu Njuri	*Cola cordifolia*	9.6	Clustered	$<10^{-6}$
	Pterocarpus erinaceus	18.3	Clustered	$9 * 10^{-6}$
	Spondias mombin	9.0	Clustered	$<10^{-6}$
	All mapped trees	7.5	Clustered	$<10^{-6}$

Discussion

1. Seedlings

The results of the randomisation tests are in accordance with the Janzen-Connell hypothesis. Seedlings of *Cola cordifolia* are more numerous but smaller in size closer to the parent tree. The only other study that has been done in tropical Africa shows similar results (Matthesius et al., 2011). They found evidence of Janzen-Connell effects in both natural and experimentally planted populations of seedlings of several species, but not for all species that have been evaluated. For certain species, it might be that slight differences in microhabitats cancel out the benefits of decreased predation away from the parent tree (Matthesius et al., 2011). For instance, seedlings that establish further away from the parent tree might be outside their preferred habitat.

Another discussion point is that light gaps could obscure Janzen-Connell effects. The reason for this is that light gaps have been shown to locally reduce the number of pathogens, which favours growth of seedlings that occur in light gaps more, compared to seedlings outside of light gaps (Molofsky & Augspurger, 1992). It would therefore be desirable to include an analysis of light gaps in the canopy for future research. Besides light gaps, other factors that could have led to the observed results include differences in leaf litter under an adult tree. Increased leaf litter has shown a reduced survival for seedlings close to the parent tree, where leaf litter is often accumulated (Molofsky & Augspurger, 1992). Another source of uncertainty comes from the relatively short window of time in which seedling data was collected. It is possible that host-specific predators were simply not present at the time of data collection (Connell, 1971).

Moreover, the results are solely based on the analysis of seedlings at two distances from the parent tree. For future studies, improvements in the methodology can be made. For a more comprehensive analysis of the distance dependence on seedling predation, taking a transect would have been preferred. This is because measuring along a transect provides a full range of values along a distance from the adult tree. Now, only two values are known, at 5 and 9 metres from the parent tree. It would have been better to have included seedling data at more distances from the parent tree, as this could have shown possible variability of the strength of Janzen-Connell effects at different distances. Also, sources of error such as GPS inaccuracy and human inaccuracy when taking measurements might have had an effect on the obtained results.

Another alternative to the method used in this study is to use larger quadrats and group seedlings into areas. For instance, Matthesius et al. (2011) used plots of 5x5 metres at several distance intervals to measure seedling abundance and height. They measured distances from 0-25 metres, using intervals of 5 metres. This might have shown a better distribution of the height and abundance of the seedlings. However, it would have been time consuming to use such a large area, as seedlings of *Cola cordifolia* were rather abundant at the time of data collection. For instance, the sample close to parent tree 1 contained 77 seedlings in 1x1 metre.

Furthermore, it would have been of interest to look more specifically at signs of predation at several distances from the parent tree. When measurements were taken, a column was left for remarks on predation, but no apparent signs of predation were observed. A more structured field protocol might have helped to systematically check for signs of predation on the seedlings. Now, seedling survival was determined based on height measurements of seedlings, where higher height indicates better survival. Including measurements on predation would have allowed for a more complete analysis of the Janzen-Connell hypothesis, as predation is hypothesised to decrease with distance from the adult tree.

2. Comparison of results for adult trees of different species

The strong tendency to show clustered patterns in all analysed species indicates a mixed result to support the Janzen-Connell hypothesis. According to Janzen (1970) and Connell (1971), predation and negative distance dependency results in regular spacing of species, which in turn promotes biodiversity. The regular spacing as a result of predation close to the parent tree should lead to a dispersed pattern. This is not observed however, as all species and the forest as a whole show clustered patterns (table 1, table 2). Based on the different results for seedlings and adult trees, it can be concluded that processes operating on the scale of seeds and seedlings around the adult tree do not necessarily operate on population scale. A similar result was observed by Schupp (1992) who did a study on Janzen-Connell effects in Panama. The explanation given is that on a very local scale, high density of seeds beneath the parent tree attracts predators, but on population level, high density of seedling patches satiates predators. Consequently, seed predators succeed at spacing out species at low densities, but not at high densities (Schupp, 1992).

Another explanation for the clustering observed is related to habitat preferences and dispersal limitation. The relatively large weight of the seeds of *Cola cordifolia* reduce the distance that seeds can travel. Because of this, seedlings that mature often do so in the vicinity of the adult tree. Interspecific competition might reduce the clustering through the life stages of the tree, from seedling, to juvenile and adult trees (Zhu et al., 2013). Therefore, spatial patterns might alter with the age of the trees. However, all adult trees that have been taken into account for the analysis were old enough to bear fruit and are consequently considered adult trees. Moreover, if seeds fall in close proximity to the parent tree, this implies that the seeds are likely located in a suitable habitat for them to establish (Zhu et al., 2013). If that is the case, it comes down to understanding the relative importance of the Janzen-Connell effects. When multiple factors come into play, it becomes challenging to point out exactly what established the observed pattern. For the Boé region, the terrain is rather heterogeneous. Even within the sacred forests, outcrops are found, and soils are rather thin. Where the soil is deep enough or cracks are formed in the bedrock, trees can establish. The lack of suitable habitat might strengthen the clustering that is observed in the studied sacred forests.

Additionally, seed dispersal by animals is likely to have an effect on seed recruitment and establishment. All tree species that were studied are consumed and dispersed by animals. Understanding the role of seed dispersers on spatial patterns of individual species would require further research, as limited studies are available.

Moreover, the results show that *Spondias mombin* trees are most strongly clustered, followed closely by *Cola cordifolia* trees. While clustering was less for the adult trees of *Pterocarpus erinaceus.* Something that might explain the difference in clustering patterns is the seed weight and dispersal of seeds. The weight of seeds of *Pterocarpus erinaceus* is a lot lower than that of *Spondias mombin* and *Pterocarpus erinaceus* seeds (Society for Ecological Restoration, 2023). The low weight of *Pterocarpus erinaceus* seeds increases the range of seed dispersal. This results in a larger distance in the nearest neighbour analysis (table 2). The weight of the seeds of *Spondias mombin* and *Cola cordifolia* is similar, which explains why their clustering is rather similar (table 2). Another reason for the observed spatial pattern has to do with the way trees regenerate. Trees do not solely reproduce from seed, but can also reproduce from underground shoots (Goba et al., 2019). For instance, Goba et al. (2019) showed from 186 samples that *Pterocarpus erinaceus* regenerates mainly from seeds (75%), but also commonly from shoots (25%), which could explain the clumping pattern that is observed for the adult trees in both Tontege and Bundu Njuri. However, the seeds are also the lightest, and thus have the capacity to travel greater distances than seeds of *Cola cordifolia* and *Spondias mombin*.

When comparing the individual species to the whole forest, rather similar results are found. This does not come as a surprise as for Tontege, the three studied tree species account for 55% of the trees in the forest. For Bundu Njuri, 72% of the forest is made up of individuals of these three species (appendix 2). In this case, the spatial distribution of the most common individual species strongly reflects the spatial pattern of the forest as a whole.

3. Future research

Firstly, more studies on Janzen-Connell effects need to be carried out on the African continent, which is currently least represented globally (Comita et al., 2014). Studies should be of a larger scale than this current study, including more species and a larger time window for data collection to account for temporal variability in predation. As soon as more studies are available, data on Janzen-Connell effects for the African continent can be included in the latitudinal gradient of biodiversity, which allows for a more holistic analysis of biodiversity worldwide.

For future research, it would be relevant to include the spatial configuration of fragmented forest patches when testing the Janzen-Connell hypothesis. One could look at the area of the fragments of forests and how isolated the fragments are from each other. This would give a better understanding of how biodiversity is maintained in fragmented habitats, whether this is natural, or caused by deforestation and other forms of land degradation. This is especially of importance in the light of increased deforestation as a result of population growth globally, and in the Boé area.

Furthermore, future studies should focus on comparing seedling establishments of multiple species, as has been done in other studies that have investigated the Janzen-Connell hypothesis (Matthesius et al., 2011). This is because the distance dependence on seedling survival has been shown to be species specific (Matthesius et al., 2011). Moreover, experimentally planting seedlings and monitoring their survival rates might help to understand the underlying factors that cause differences in seedling survival between species.

Finally, looking more closely at the role of seed dispersers for seedling recruitment, and how this relates to the Janzen-Connell effect would be of interest to study. If seedlings are predominantly animal dispersed, this might affect the structuring of the forest and the biodiversity. This is of relevance as the animals that disperse the seeds are often threatened species, such as the Western Chimpanzee. Therefore, the decline of these species also affects the way that seeds are dispersed and recruited, and the way that diversity is maintained.

Conclusion

In summary, while the obtained results are mixed and limitations in the data collection exist, Janzen-Connell effects were observed for seedlings in two sacred forests of the Boé. Seedlings show an increase in height and a decrease in abundance away from the parent tree, as proposed by the Janzen-Connell hypothesis. For the adult trees, there was no support for the Janzen-Connell hypothesis, as all the studied species showed clustered patterns in the spatial analysis. These patterns could have been caused by something else than the Janzen-Connell effects, such as habitat preferences. This implies that processes operating on the scale of seeds and seedlings around the adult do not necessarily need to operate on population scale. Consequently, more studies need to be done to better understand the drivers of biodiversity in Africa and the Boé. The natural heritage of the Boé is a refuge for biodiversity and threatened species, which needs to be protected and conserved for future generations.

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Appendix

1. Field protocol

A local guide from the Chimbo staff will help with locating the five sacred forest sites and the identification of trees and seedlings. The GPS and knowledge of the local guide will be used to keep track of trees that have been recorded, so that no duplicates are produced.

Parent trees for seedling samples are selected so that two trees are not too close together, to avoid confusion of the origin of the seeds from the two trees. This means that crowns cannot overlap. This is assessed visually for each site.

The following guidelines were used for the measurements:

Parameter to measure	Materials	Method	Other
GPS coordinates [decimal degrees] of parent tree and sampling sites for seedlings	GPS	The GPS marks points in the map. A number will be assigned for each individual tree to match the location which are displayed in decimal degrees (dd,ddddd).	Coordinate system: WGS84
Average crown diameter parent tree [m2]	Clinometer, measuring tape	Use the clinometer to stand right under the edge of the crown. Use measuring tape to measure distance between extremities. Do this for the longer and shorter side perpendicular to each other.	
Height seedlings [cm]	Measuring tape	Measure from the ground to the highest point of the seedling. This could be either the stem or the leave.	
Abundance of seedlings	Quadrat	Count all the *Cola cordifolia* seedlings within the quadrat. Only seedlings that are fully in the quadrat are included in the count.	

2. Species inventory for five sacred forests

Table 3. Table of recorded species in the five sacred forests and their abundance. The three most common tree species have been highlighted in orange. These are also the three species that have been included in the data analysis.

Tree species	Fula name	Bundu Njuri	Quebube	Tontege	Patte Patte	Lugajole	Total
Vitex spp.	Bumel Bape	15	3	9	2	1	30
Lannea velutina	Chuko	0	11	1	32	3	47
Pterocarpus erinaceus	Bani	50	45	52	99	28	274
Parkia biglobosa	Nete	22	28	13	26	2	91
Spondias mombin	Tchale	63	9	250	110	21	453
Hexalobus monopetalus	Boile Kunje	3	19	40	20	0	82
Cola cordifolia	Taba	77	2	269	31	10	389
Parinari excelsa	Kura	1	3	14	6	0	24
Cordyla pinnata	Duke	0	1	0	0	0	1
Sorindeia juglandifolia	Sangi Bombo	2	4	144	38	64	252
Landolphia heudelotii	Pore Paje	1	4	13	8	4	30
Voacanga africana	Boile Bonoro	0	0	0	1	1	2
Dialium guineensis	Meko	6	14	49	45	43	157
Ceiba pentendra	Bantang	0	3	8	5	7	23
Sarcocephalus latifolius	Dunduke	1	1	1	24	6	33
Strychnos spinosa	Pata Kule	1	1	0	0	0	2
Pachystela pobeguiniana	Buna Dodi	6	2	60	52	15	135
Piliostigma thonningii	Barke	1	1	5	26	0	32
Detarium senegalense	Boto	0	1	9	4	5	19
Uvaria chamae	Boile Banana	0	0	6	5	2	13
Daniellia oliveri	Tchewe	0	1	0	0	0	1
Saba senegalensis	Lare	3	4	42	30	27	106
Milicia regia	Chime	0	0	0	0	2	2
Anisophyllea laurina	Kansi	0	0	0	0	1	1
Treculia africana	Gilinti	0	0	8	4	0	12
Allophylus africanus	Cor Ndeyala	5	0	2	24	1	32
Bombax costatum	Lukun	2	0	2	21	0	25
Khaya senegalensis	Kai	1	0	3	0	0	4
Pseudospondias microcarpa	Ghara	0	0	2	0	0	2
Ficus sur	Ibe	0	0	2	7	0	9
Afzelia africana	Lenge	3	0	31	9	0	43
Mangifera indica	Mango	0	0	1	0	0	1
TOTAL TREES		263	157	1036	629	243	2327